PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:

SKOULTCHI

Serial No.: 08/102,390 — Group Art Unit: 1804

Filed: August 5, 1993 — Examiner: Ziska, S.

For: PRODUCTION OF PROTEINS USING HOMOLOGOUS RECOMBINATION — Atty Docket No.: 7639-017/Cell 3.2

REQUEST UNDER 37 C.F.R. §1.607
FOR INTERFERENCE WITH A PATENT

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

Pursuant to 37 C.F.R. §1.607, the Applicant hereby seeks to have an interference declared between the above-identified application and United States Patent No. 5,272,071 granted to Chappel ("'071 Chappel patent") entitled "Method for the Modification of the Expression Characteristics of an Endogenous Gene of a Given Cell Line" (Exhibit A).

The '071 Chappel patent issued December 21, 1993 from application Serial No. 893,447 ("'447 parent application") filed May 28, 1992 as the national stage of PCT application no. PCT/US90/07642 filed December 21, 1990. The '447 application claims priority under 35 U.S.C. §120 as a

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RICHARD BORNSTEIN
(Type or print name of person mailing paper or fee)

(Signature of person mailing paper or fee)

continuation-in-part of application Serial No. 07/454,783 filed December 22, 1989, now abandoned ("Chappel grandparent application"). The Chappel grandparent application is attached hereto as Exhibit B.

The '071 Chappel patent is assigned to Applied Research Systems, Ars. Holding N.V., Curacao, Netherlands, Antilles.

The instant application is a continuation of application Serial No. 07/787,390 ("'390 parent application") filed November 4, 1991 as the national stage of PCT Application No. PCT/US90/06436, filed November 6, 1990. The '390 parent application claims priority under 33 U.S.C. §120 as a continuation-in-part of application Serial No. 432,069 filed November 6, 1989 ("'069 grandparent application"). The Applicant's '069 grandparent application is attached hereto as Exhibit C.

The instant application, including the '390 parent and '069 grandparent applications are assigned to Cell Genesys, Inc.

In this Request, Applicant proposes a single count directed to the use of targeted homologous recombination to integrate a regulatory element and/or an amplifiable gene into a host cell genome within or proximal to a gene of interest which is endogenous to the host cell in order to activate or modify expression of the gene of interest. Claims 1 to 58 of the '071 patent should be designated as corresponding to the proposed count. Claims 26-43, 48-61 and 67-81 and 86-96 of

the instant application should be designated as corresponding to the proposed count.

I. GENE EXPRESSION VIA TARGETED HOMOLOGOUS RECOMBINATION

The present application discloses, inter alia, the use of targeted homologous recombination to achieve activation and/or enhanced expression of target genes endogenous to mammalian host cells. Targeting vectors are designed and used to integrate a regulatory element and/or an amplifiable gene into a mammalian host cell genome at a location within or proximal to the target gene of interest. The integration of a regulatory element so that it is operatively associated with the target gene results in gene activation and/or enhanced or modified expression; i.e., the integrated regulatory element will drive the expression of the target gene in the host cell. Where an amplifiable gene is integrated at a location within or proximal to the target gene, the target gene can be amplified by applying the appropriate selection pressure so that expression of the target gene is enhanced.

As discussed in detail in the Amendment Under 37 C.F.R. § 1.115 submitted herewith, the claims of the instant application are entitled to the November 6, 1989 filing date of the '069 grandparent application and are allowable over the prior art. The asserted Chappel PCT application (WO91/09955) was published July 11, 1991, well after the effective date of the instant application, and therefore, is not prior art. Likewise, the Chappel '071 patent is not prior art, because

the November 6, 1989 effective date of the instantly pending claims antedates the earliest effective date to which the Chappel '071 patent could possibly be entitled; i.e., assuming arguendo, the '071 patent were entitled under 35 U.S.C. § 112 and § 120 to the December 22, 1989 filing date of its grandparent application.

II. THE PROPOSED COUNT

Applicant proposes the following count:

Proposed Count

> A method of activating the expression of a normally transcriptionally silent target gene or modifying the expression characteristics of a target gene within the genome of a cell line, comprising inserting a DNA construct into said genome by homologous recombination, said DNA construct comprising (a)(i) a DNA regulatory segment which when operatively linked to the target gene stimulates expression of a silent target gene, or modifies the expression characteristics of the target gene as compared to its existing DNA regulatory segment, or (ii) an amplifiable gene capable of amplifying said target gene when inserted in sufficiently close proximity thereto, and (b) a DNA targeting segment homologous to a region of said genome within or proximal to said target gene, wherein said construct is inserted such that said regulatory segment or amplifiable gene is operatively linked to said target gene of interest.

The proposed count is substantially identical to Claims 1, 2 and 3 of the '071 patent; i.e., Claim 1 specifies activating the transcription of a normally silent target gene in a cell line by integrating a regulatory element that activates the silent gene; whereas Claims 2 and 3 specify modifying the expression characteristics of the endogenous

target gene in a cell line by integrating a regulatory element (Claim 2) or amplifiable gene (Claim 3). Claims 2 and 3, which cover modifying, e.g., enhancing, the expression of a target gene are obvious variants of Claim 1 which covers activating an otherwise transcriptionally silent gene. Therefore, Claims 1, 2 and 3 which correspond to the proposed count are directed to the same patentable invention. This proposed count was provided because Rule 606 states that at the time an Interference is initially declared, a count shall not be narrower in scope than any patent claim which corresponds to the count.

III. CLAIMS 1-58 OF THE '071 CHAPPEL PATENT CORRESPOND TO THE PROPOSED COUNT

The Applicant proposes that Claims 1-58 of the '071 Chappel patent correspond to the proposed count. Claims 1, 2 and 3 are substantially identical to the proposed count and, therefore, correspond exactly to the proposed count. Claims 4-58 do not correspond exactly to the proposed count, but such claims are directed to the same patentable invention as the proposed count. 37 C.F.R. §1.601(n).

A. Method Claims

Claim 1 covers methods for activating a transcriptionally silent target gene in a cell line using homologous recombination to integrate a regulatory element capable of stimulating expression of the target gene into the host cell genome at a location within or proximal to the

target gene. Claim 2 covers methods for modifying the expression characteristics of a target gene in a cell line using homologous recombination to integrate a regulatory element capable of modifying the expression characteristics of the gene into the host cell genome within or proximal to the target gene. Claim 3 covers methods for modifying the expression characteristics of a target gene in a cell line using homologous recombination to integrate an amplifiable gene into the host cell genome sufficiently proximal to the target gene to cause amplification of the target gene. Each of Claims 1, 2 and 3 corresponds exactly to the proposed count.

Claims 5-12, 28-35 and 40-47 depend from Claims 1, 2 and 3, respectively, and correspond substantially to the proposed count. Claims 5-8 specify that the DNA construct used in the method of Claim 1 includes two targeting segments homologous to portions of the host cell genome (Claim 5), a selectable marker (Claim 6), a negative selectable marker (Claim 7), or an amplifiable gene (Claim 8) in addition to the regulatory element. Claims 9-12 specify that the cell line used in the method of Claim 1 is eukaryotic (Claim 9), animal (Claim 10), mammalian (Claim 11), or plant (Claim 12). Claims 28-35 and Claims 40-47 are identical to Claims 5-12 except that they depend from Claims 2 and 3, respectively.

The use of the targeting sequences, selectable markers, amplifiable genes, and/or host cell lines specified in Claims 5-12, 28-35 and 40-47 do not provide a basis for

patentable distinction over the proposed count because these additional elements were well known choices in the art of gene expression. Therefore, Claims 5-12, 28-35 and 40-47 define the same patentable invention as the proposed count, and should be designated as corresponding to the proposed count.

Claims 13-16, 36-39 and 48-51 ultimately depend from Claims 1, 2 and 3, respectively, and correspond substantially to the proposed count. Claim 13 covers methods for recovering the expressed gene products from host cells having an integrated regulatory element that activates a silent gene (Claim 1) and an integrated selectable marker (Claim 6). In particular, Claim 13 specifies that the method includes selecting cell lines that express the integrated selectable marker, culturing the selected clones under conditions that permit expression of the target gene product, and collecting the target gene product from the culture. Claims 14-16 depend from Claim 13 and further specify the use of the neomycin resistance gene as the selectable marker (Claim 14), the use of a negative selectable marker (Claim 15), and the use of the herpes virus thymidine kinase (TK) gene as the negative selectable marker (Claim 16). Claims 36-39 and Claims 48-51 are identical to Claims 13-16 except that they depend from Claims 2 and 3, respectively.

The recovery of the expressed gene product from cultures of cell lines engineered to contain selectable markers does not provide a basis for patentable distinction over the proposed count, because there is no other utility for

the method of the proposed count. Thus, Claims 13-16, 36-39 and 48-51 define the same patentable invention as the proposed count and should be designated as corresponding to the proposed count.

B. Cell Line Claims

Claims 18-24 cover host cell lines necessary to practice the method of the proposed count and, therefore should be designated as corresponding to the proposed count.

In particular, Claim 18 covers cell lines capable of expressing a normally transcriptionally silent gene, in which the cell line has an integrated regulatory element linked to the gene. Claims 22-24 depend from Claim 18, defining the regulatory element as one capable of promoting expression of a gene product normally expressed by the cell line (Claim 22), and further include an inserted selectable marker (Claim 23), and an inserted amplifiable gene (Claim 24). Claims 19-21 cover cell lines having an exogenous regulatory element or amplifiable gene that enhances expression of the target gene.

Host cell lines in which a normally silent gene has been activated or gene expression is enhanced are not believed to be separately patentable over the method of the proposed count, since such host cell lines are necessary to practice the method of the proposed count. In addition, the only utility of the host cell lines of Claims 18-24 is for use in the method of the proposed count. Therefore, Claims 15-24 should be designated as corresponding to the proposed count.

Claims 25 and 53-58 correspond substantially to the proposed count. Claims 25 and 56-58 specify culturing the cell lines of Claims 18 and 22-24 under conditions which permit expression of the gene product and collecting the gene product from the culture. Claim 25 specifies that the cell line is a differentiated cell line. Claims 53-55 are identical to Claims 56-58, except that they specify culturing the cell lines of Claims 19-21.

The only utility of the method of the proposed count is to express and collect the target gene product from cultures of the engineered host cells. Therefore, the method of Claims 25 and 53-58 define the same patentable invention as the proposed count, and should be designated as corresponding to the proposed count.

C. **DNA Claims**

Claims 26 and 27 correspond substantially to the proposed count. These claims cover DNA constructs used in the targeted homologous recombination method for integrating a regulatory element into a host cell genome so that it is operatively linked to the target gene (Claim 26), or for integrating an amplifiable gene in close proximity to the target gene (Claim 27). DNA constructs containing such targeting sequences are not believed to be separately patentable over the method of the proposed count, since such DNA is necessary to practice the method of the proposed count.

Claim 17 is an independent claim covering the recombinant genome of a cell line having an integrated regulatory element operatively linked to the target gene. Claim 17 corresponds substantially to the proposed count -- the only utility for the recombined genome of Claim 17 is for use in the method of the proposed count.

IV. CLAIMS 26-43, 48-61, 67-81 and 89-96 OF THE INSTANT APPLICATION CORRESPOND TO THE PROPOSED COUNT

The Applicant's Claims 26-43, 48-61, 67-81, and 89-96 do not correspond exactly to the proposed count, but all of these claims, should be designated as corresponding to the proposed count, because they are directed to the same patentable invention as the proposed count. As discussed in Section V, infra, the remaining Claims 44, 46, 47, 62-66, 82-88 and 97-104 cover a separately patentable invention, and should not be designated as corresponding to the proposed count.

A. Method Claims

Claims 48-61 and 75-81; 67-68 and 89-90; and 69-70 and 91-96 cover methods for producing mammalian host cells and the target gene product via targeted homologous recombination.

In particular, Claim 48 covers methods for producing a mammalian host cell having a target gene modified by the integration, via homologous recombination, of a regulatory element into the genome of the host cell in operative association with the target gene, so that the regulatory

element controls expression of the target gene. Claim 67 is identical to Claim 48 but specifies that the modified gene is activated by the integrated regulatory element. Claim 49 covers methods for producing a mammalian host cell having an amplifiably modified target gene, using homologous recombination to integrate an amplifiable gene into the appropriate location within the host cell genome. Claims 75 and 76 specify the use of both an integrated regulatory element and an amplifiable gene. Each of Claims 48, 49, 67, 75 and 76 corresponds exactly to the proposed count.

Claims 50-61 and 77-81 which depend from Claims 48 and 49, and Claims 68 and 89-90 which depend from Claim 67, specify promoters/enhancers, selectable markers, mammalian host cells, amplifiable genes and mutated target genes that can be used in the claimed methods, and correspond substantially to the proposed count.

In particular, Claim 50 specifies that the method of Claim 48 or 49 includes the use of a selectable marker. Dependent claims 79-81 specify the markers that can be used.

Claims 51-56 and 68 specify that the type of mammalian host cell used in the method of Claim 48 or 49 is a primary cell that does not grow readily in culture (Claim 51); a primate, human, normal/neoplastic, somatic/germ cell, or dipoloid fibroblast cell (Claims 52-56); or a cell line, e.g., CHO, monkey kidney cell line, 3T3 cells, Vero cells or 293 cells (Claim 68).

Claim 57 specifies that the amplifiable gene used in the method of Claim 49 or 75 includes dihydrofolate reductase (dhfr), metallothionine genes, adenosine deaminase, glutamine synthetase, or ornithine decarboxylase.

Claims 58-61 specify that the method of Claim 48 or 49 includes the use of homologous recombination to mutate the activated and/or amplified target gene (Claim 58), e.g., in the coding region (Claim 59), in the 3' untranslated region (Claim 60), or in the 5' region (Claim 61).

Claims 77-78 specifies the promoters/enhancers that can be used as regulatory elements in the method of claims 48 and 76.

The use of the selectable markers, mammalian host cells, particular amplifiable genes, and/or promoters/ enhancers and mutated genes of interest specified in Claims 51-61, 68, 75-81 and 89-90 do not provide a basis for patentable distinction over the proposed count, because these particular specified elements were well known choices in the art of gene expression. Therefore, Claims 51-61, 68, 75-81 and 89-90 define the same patentable invention as the proposed count and should be designated as corresponding to the proposed count.

Claims 69, 70 and 91-96 cover methods for producing the target gene product expressed by the engineered host cells, and correspond substantially to the proposed count. In particular, Claim 69 specifies culturing mammalian host cells containing an integrated regulatory element operatively linked

to the target gene, under conditions which permit expression of the target gene. Claim 91 specifies the use of an amplifiable gene integrated within or proximal to the target gene. Claims 91 and 93 specify the use of both an integrated regulatory element and an amplifiable gene. Dependent claims 95-96 further specify that the regulatory element is a promoter, enhancer, and in particular, the CMV promoter/ enhancer. Claim 70 and 94 further specify recovering the target gene product from the cultured mammalian cell.

The production and recovery of the expressed gene product from cultures of the host cells engineered via homologous recombination does not provide a basis for patentable distinction over the proposed count, because there is no other utility for the method of the proposed count. Thus, Claims 69, 70 and 91-96 define the same patentable invention as the proposed count and should be designated as corresponding to the proposed count.

B. **Host Cell Claims**

Claims 26-43 and 72-74 cover mammalian host cells necessary to practice the method of the proposed count, and therefore, should be designated as corresponding to the proposed count.

In particular, Claim 26 covers a mammalian host cell containing a target gene modified by the integration, via homologous recombination, of a regulatory element different from the wild-type regulatory element normally associated with

the target gene into the genome of the host cell, so that the regulatory element controls expression of the target gene. Claim 32 covers a mammalian host cell having an amplifiable gene integrated via homologous recombination into the host cell genome within or proximal to the target gene so that the target gene is also amplifiable. Claims 27 and 72 specify that the host cell contains both an integrated regulatory element and an amplifiable gene.

Claims 28-31, 33-43, and 73-74 which depend from Claims 26, 27, 32, and 72 specify promoter/enhancer regulatory elements, amplifiable genes, mutated target genes and the types of mammalian host cell, and correspond substantially to the proposed count.

In particular, Claims 28-31 depend from Claim 26 or 27 and further specify mutations in the target gene (Claim 28); e.g., in the coding region (Claim 29), the 3' untranslated region (Claim 30), or in the 5' region (Claim 31). Claims 33-36 are identical to Claims 28-31 but depend rom Claim 32 or 72.

Claims 37-42 depend from Claims 26, 27, 32 or 72 and specify that the mammalian host cell is a primary cell which does not grow readily in culture (Claims 37), or the mammalian cell type; e.g., primate, human, normal/neoplastic, somatic/germ cell, or diploid fibroblast (Claims 38-42).

Claim 43 depends from Claim 27 or 32 and specifies that the amplifiable gene includes dhfr, metallothionine

genes, adenosine deaminase, glutamine synthetase or ornithine decarboxylase.

Claims 73-74 depend from Claim 26 or 72 and specify various promoters/enhancers as the regulatory element.

The mammalian host cells covered by the foregoing claims are not believed to be separately patentable over the method of the proposed count, since such host cells are necessary to practice the method of the proposed count. In addition, the only utility of the mammalian host cells of Claims 26-43 and 72-74 is for use in the method of the proposed count. Therefore, Claims 26-43 and 72-74 should be designated as corresponding to the proposed count.

V. CLAIMS 44, 46-47, 62-66 82-88 and 97-104 OF THE INSTANT APPLICATION DO NOT DEFINE THE SAME PATENTABLE INVENTION AS ANY CLAIM THAT CORRESPONDS TO THE PROPOSED COUNT AND THEREFORE SUCH CLAIMS SHOULD NOT BE DESIGNATED AS CORRESPONDING TO THE PROPOSED COUNT

Claims 44, 46, 47, 62-66, 82-88 and 97-104 cover transferring the activated/amplifiable target gene, which was engineered by homologous recombination in the primary mammalian host cells, to a secondary expression host cell which has superior growth characteristics, and the use of the secondary host cells to produce the target gene product. For reasons detailed below, these claims are not obvious variants and are separately patentable over the claims corresponding to the count, and therefore, should not be designated as corresponding to the count.

In particular, Claim 62 covers methods for producing the secondary expression host cells using DNA derived from a mammalian host cell containing an integrated regulatory element that controls expression of the target gene, whereas Claim 63 covers such methods using an integrated amplifiable gene. Claims 82 and 83 cover the method using both an integrated regulatory element and an amplifiable gene.

Claims 64-66 and 84-88 depend from Claims 62, 63, 82 and 83 and further specify the use of a selectable marker (Claims 64 and 86-88), and secondary host cells that are mammalian (Claim 65); e.g., CHO cells, monkey kidney cells, CHO cells, 3T3 cells, Vero cells, or 293 cells (Claim 66); and promoter/enhancers (Claims 84-85).

Claim 44 covers secondary host cells containing the target gene controlled by the integrated regulatory element and/or the amplifiable gene derived from the mammalian cell engineered via homologous recombination. Claims 46 and 47 depend on Claim 44 and further specify that the secondary expression host cell is mammalian (Claim 46); e.g., CHO, monkey kidney cells, C127 cells, 3T3 cells, Vero cells or 293 cells (Claim 47).

Claims 97-104 cover culturing the secondary host cell containing the target gene controlled by the integrated regulatory element (Claim 97), an amplifiable gene (Claim 100) or both (Claims 98 and 101), and include the isolation of the target gene product from the secondary host cell culture

(Claims 99 and 102). Claims 103 and 104 specify the promoters/enhancers used.

Claims 44, 46-47, 62-66, 82-88 and 97-104 do not define the same patentable invention as any claim that corresponds to the proposed count. The claims that correspond to the proposed count do not suggest the improvement afforded by the transfer of the activated/modified target gene to a secondary expression host. This improvement allows one to express gene products by performing targeted homologous recombination on host cells that contain the gene of interest, e.g., a human gene, but which are unsuitable or unusable for long-term or large-scale culture. For example, in the invention covered by Claims 44, 46-47, 62-66, 82-88 and 97-104, a target gene known to be located in a primary host cell which is not a continuous cell line can be activated/modified and then transferred to a secondary expression host cell which can be cultured on a large scale. Unlike the claims corresponding to the count, in this system, the target gene is exogenous to the secondary host cell. Therefore, Claims 44, 46-47, 62-66, 82-88 and 97-104 should not be designated as corresponding to the proposed count.

VI. THE APPLICANT HAS COMPLIED WITH 35 U.S.C. §135(b)

The Applicant has complied with 35 U.S.C. § 135(b) in that the present application is claiming the same invention as claimed in the '071 patent within one year from the December 21, 1993 date on which the '071 patent issued.

VII. APPLICANT'S EFFECTIVE FILING DATE ANTEDATES THE EARLIEST FILING DATE WHICH COULD POSSIBLY BE ACCORDED TO THE '071 CHAPPEL PATENT

For reasons set forth in more detail in the accompanying Amendment Under 37 C.F.R. §1.115, the Applicant is entitled to the benefit of the November 6, 1989 filing date of the '069 grandparent application because the '069 application meets the written description and enablement requirements of 35 U.S.C. § 112, first paragraph, of at least one species within the proposed count. Squires v. Corbett, 560 F.2d 424, 433 (C.C.P.A. 1973) and Weil v. Feitz, 572 F.2d 856, 865-6 n. 16 (C.C.P.A. 1978).

The '069 grandparent application clearly describes the use of targeted homologous recombination to integrate a regulatory element and an amplifiable gene into a host cell genome within or proximal to a target gene in order to activate and/or enhance expression of the target gene product (e.g., see '069 grandparent application at Summary of the Invention, p. 3; and Description of the Specific Embodiments, pp. 3-11).

Accordingly, since the '069 application meets the requirements of Section 112, first paragraph with respect to the proposed count, the Applicant is entitled to the benefit of its November 6, 1989 filing date of the '069 application for the proposed count.

The '071 Chappel patent issued from a series of applications, the earliest of which was filed December 22, 1989. Even assuming, arguendo, that the '071 Chappel patent

were entitled to the benefit of the December 22, 1989 filing date under 35 U.S.C. § 112, the Applicant's effective date antedates the earliest effective date to which the '071 Chappel patent could possibly be entitled. Thus, there is a basis upon which applicant is entitled to judgment relative to the patentee, and no affidavit under 37 C.F.R. § 1.608(a) or evidence under 37 C.F.R. § 1.608(b) is required to provoke the requested interference. See M.P.E.P. § 2308.03.

VIII. CONCLUSION

The Applicant seeks declaration of an interference in which there is one count relating to the targeted homologous recombination to achieve gene activation and/or modified or enhanced expression of a gene product. The Applicant's Claims 26-43, 48-61, 67-81 and 89-96, and Claims 1-58 of the '071 Chappel patent should be designated as corresponding to the proposed count.

The declaration of an interference is earnestly solicited.

Respectfully submitted,

Date: June 1, 1994

30,742
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Enclosures:
Exhibit A - U.S. Patent No. 5,272,071
Exhibit B - Chappel Grandparent Application Serial No. 07/454,783 filed December 22, 1989
Exhibit C - Applicant's '069 Grandparent Application Serial No. 432,069 filed November 6, 1989